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USSR Report

LIFE SCIENCES

EFFECTS OF NONIONIZING ELECTROMAGNETIC RADIATION

(FOUO 2/81)

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CONTENTS

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STUDY OF SHF MICROWAVE EFFECT ON SEX AND SOMATIC CELLS OF MAMMALS

Kiev TSITOLOGIYA I GENETIKA in Russian Vol 14, No 6, Nov-Dec 80
(manuscript received 7 Feb 79) pp 3-8

/Article by L. K. Ramayya, M. D. Pomerantseva, G. A. Vilkina and V. S. Tikhonchuk, Institute of General Genetics of the USSR Academy of Sciences, Moscow/

/Text/ Introduction. Many studies showing the harmful effect of SHF electromagnetic waves on the reproductive system and its function in mammals have appeared recently /1-3/. As yet, however, the problem of the genetic effect of SHF range microwaves remains little studied. The data on the mutagenicity of a SHF electromagnetic field available in the literature are contradictory. There are data on a weak mutagenic effect of SHF microwaves during a fractionated irradiation of bone marrow cells /4, 5/ and sex cells of male mice /6, 7/. At the same time, other studies did not reveal changes in the properties of nucleic acids of the spermatogenic epithelium /8/ or a mutagenic effect under the effect of SHF microwaves on yeast cells /9/. The results of studies of the combined effect of SHF microwaves, chemical mutagens and radiation are difficult to compare and often contradictory /10, 11/.

Material and methods. The genetic effect of a single and fractioned action of SHF range microwaves with energy flux density of 60 and 800 mW/cm^2 and a wave length of 12.6 cm (oscillation frequency 2,400 MHz) was studied. This range encompasses the upper and lower limits of the values of energy of a SHF electromagnetic field most frequently used in practice. Hybrid sexually mature male mice F_1 (CBAXC57BL) were subjected to the action. Nonline white females were used in the variant with a single action and both white nonline and hybrid females aged 2.5 to 4 months, in the variant with a fractionated action. Every case had its own biological identical control, which ruled out the effect of the female genotype on embryonal mortality.

To detect the mutagenic effect of SHF microwaves, three tests were used: frequency of dominant lethal mutations in sex cells of male mice; frequency of anomalous spermium heads and frequency of chromosome aberrations in bone marrow cells.

Males were irradiated on the Parus unit in an anechoic chamber under a single action for 12 min with energy flux density of 60 mW/cm^2 and for 21 s with energy flux density of 800 mW/cm^2. The time of irradiation was chosen so that the level of lethal effect of microwaves did not exceed 10%. In this investigation the mortality of animals depending on the series of experiments ranged from 0.1 to 10%.

Under a fractionated action the conditions of irradiation were as follows: energy flux density 800 mW/cm^2 and time of irradiation 21 s once a day for 10 days at an ambient temperature of 21-22 °C.

To detect dominant lethal mutations, intact females were exposed to irradiated males for a week. Under a single effect the succession of six implantations made guaranteed participation in fertilization of gametes irradiated at the stage of mature spermia (first week), late spermatids (second week), early spermatids (third week), late spermatocytes (fourth week), early spermatocytes (fifth week) and spermatogonia (sixth week). In case of a fractionated action irradiation lasted 10 days. Therefore, spermia irradiated at the stages of spermia and spermatids (first week), spermatids and spermatocytes (second and third weeks), spermatocytes (fourth week) and spermatogonia (fifth week) took part in fertilization. The number of implantations in this variant was five. Eighteen days after the beginning of crossing the pregnant females were anesthetized and cut open. The percent of embryonal death was determined on the basis of the ratio of the number of yellow bodies in the ovary, places of implantation and live embryos in the uterus.

The frequency of induced dominant lethal mutations was determined on the basis of a comparison of the following indicators: survival rate of embryos (ratio of the number of live embryos to the number of yellow bodies); death before implantation (ratio of the difference between the number of yellow bodies and places of implantation to the number of yellow bodies); mortality of embryos after implantation (ratio of the number of dead embryos to the number of places of implantation) /12/. Furthermore, the percent of effective crossings and the weight of testes 45 days after the action were determined. These indicators made it possible to judge the effect of SHF microwaves on the reproductive capacity of the males.

To count anomalous spermium heads, smears from the content of the epididymis were prepared on the 35th day after the action. The frequency of anomalous heads of mature spermia developed from irradiated spermatogonia attests to the mutagenicity of the action factor /13/. No less than 300 spermatozoids from each of the six males were investigated.

Chromosome disturbances in bone marrow cells were analyzed in metaphases on permanent preparations /14/ on completion of a 10-day fractionated irradiation.

It should be noted that standard methods of statistical analysis of the data on dominant lethal mutations have not been developed conclusively to this day /15/. The method χ^2 for an evaluation of the differences in postimplantation mortality between the experiment and control was used in this work. Another method of statistical processing was used in some cases /16/. According to this method the evaluation of errors was calculated on the basis of the following formula:

$$\frac{\Sigma y_i}{\Sigma x_i}\sqrt{\frac{n}{n-1}\left[\frac{\Sigma (y_i-\bar{y})^2}{(\Sigma y_i)^2}+\frac{\Sigma (x_i-\bar{x})^2}{(\Sigma x_i)^2}-2\frac{\Sigma (x_i-\bar{x})\Sigma (y_i-\bar{y})}{(\Sigma x_i)(\Sigma y_i)}\right]}$$

where n is the number of investigated females; x_i is the number of yellow bodies; y_i is the number of embryos in some females; $\bar{x}$, $\bar{y}$ are the corresponding average values calculated for all females.

The evaluation of the significance of differences was made according to Student's t-criterion.

Results of investigations and their discussion. Table 1 presents data on embryonal death caused by genetic disturbances arising in sex cells of males as a result of a single action of SHF microwaves. As can be seen from table 1, the average number of yellow bodies, places of implantation and live embryos per female did not differ significantly between the experiment and control. Nor did we find statistically significant differences between the experiment and control in postimplantation mortality, which, as is well known, is the basic indicator of the frequency of dominant lethal mutations. We would like to note that nongenetic factors, such as the level of fertility of spermia, the number of ovulated ova and so forth, can also have an effect on the amounts of preimplantation losses and, accordingly, the survival rate of embryos /12/. A certain increase in postimplantation mortality observed under the effect on late spermatids (fourth week) proved to be statistically insignificant ($\chi^2=2.4$; $P>0.05$). Thus, induced dominant lethal mutations were not detected at any stage of spermatogenesis.

The percent of effective crossings in the experimental groups of mice was even slightly higher than the control level. Forty-five days after the action the weight of testes did not differ from the control level. In control and experimental groups it averaged 207, 210 and 213 mg respectively.

Table 2 presents the results of analysis of embryonal mortality in the offspring of males subjected to a fractionated irradiation. As follows from the data, in this experimental variant, as well as under a single action, there were no differences between the experiment and control in the average number of yellow bodies, places of implantation and live embryos per female. Under the effect on gametes realized during the first 2 weeks after processing a small increase in postimplantation mortality in the experiment was observed. In one case the differences between the experiment and control proved to be statistically significant ($\chi^2=5.2$; $P<0.05$). However, it should be noted that, since the method χ^2 does not make it possible to take into consideration the individual differences among females, in this case we additionally used another method of statistical data processing /16/, which did not confirm the statistical significance of the observed differences ($t=0.4$; $P>0.5$). If the data on the combined effect of SHF microwaves on all the stages of spermatogenesis are presented, as follows from table 2, the lack of a mutagenic effect can be seen clearly.

Under a fractionated action of SHF microwaves disturbances in the form of spermium heads were studied. According to the data in the literature, the period of 35 days is optimal for the use of this indicator during a study of the effect of ionizing radiation on spermatogenesis, because the largest number of anomalous heads is observed during this period. It is assumed that anomalous spermium heads are formed as a result of genetic disturbances of the type of point mutations in sex cells subjected to some mutagenic action /13/. As an analysis of the preparations showed, the frequency of anomalous spermium heads in the experiment and control was on the same level and averaged 1.4 and 1.6% respectively.

Table 1. Embryonal Mortality in the Offspring of Males Subjected to a Single Action of SHF Microwaves

(1) Сроки спаривания, недели	(2) Вариант воздействия	(3) Количество подсаженных ♀	(4) Проц. эффективных скрещиваний	(5) Желтых тел на ♀	(6) Мест имплантации на ♀	(7) Живых эмбрионов на ♀	(8) Выживаемость, проц.	(9) Смертность: (10) до имплантации, проц.	(9) Смертность: (11) после имплантации, проц.
(12) 1-я	СВЧ-I*(13)	36	83,3	9,5	8,3	7,8	81,8	13,0	6,0
	(13) СВЧ-II**	36	97,2	8,9	8,2	7,3	82,0	8,0	10,8
	(14) Контроль	36	83,3	9,7	8,8	7,8	80,5	9,2	11,3
(15) 2-я	(13) СВЧ-I	36	91,7	9,8	8,9	8,2	83,7	9,5	7,5
	СВЧ-II(13)	36	97,2	9,8	9,2	8,5	86,8	5,9	7,8
	(14) Контроль	36	80,6	9,6	8,9	8,3	86,0	7,9	6,6
(16) 3-я	СВЧ-I	35	94,3	10,1	8,6	7,8	77,8	14,4	9,1
	(13) СВЧ-II	36	86,1	9,7	8,7	7,8	80,1	10,9	10,0
	(14) Контроль	36	77,8	10,5	9,6	8,6	82,0	8,5	10,4
(17) 4-я	СВЧ-I	36	86,1	10,6	10,0	9,1	85,4	6,4	8,7
	(13) СВЧ-II	36	69,4	10,8	10,1	8,6	80,3	5,9	14,6
	(14) Контроль	36	80,6	10,1	9,2	8,3	82,2	8,9	9,8
(18) 5-я	(13) СВЧ-I	36	69,4	10,5	9,8	9,3	88,9	6,1	5,3
	СВЧ-II	36	83,3	10,5	9,7	8,7	83,4	7,6	9,7
	(14) Контроль	36	50,0	9,9	9,6	8,6	86,6	3,4	10,4
(19) 6-я	(13) СВЧ-I	36	72,2	9,9	9,2	8,5	85,6	6,6	8,3
	СВЧ-II	33	66,7	9,9	9,3	8,4	84,9	6,4	9,3
	(14) Контроль	29	58,6	9,8	9,1	8,4	85,6	7,2	7,7
(20) Всего	СВЧ-I	215	82,8	10,1	9,1	8,4	83,6	9,5	7,6
	(13) СВЧ-II	213	83,6	9,8	9,1	8,2	83,0	7,5	10,3
	(14) Контроль	209	72,2	10,0	9,2	8,3	83,4	7,9	9,5

*Super high frequency-I--energy flux density--60 mW/cm^2, irradiation 12 min. **Super high frequency-II--energy flux density--800 mW/cm^2, irradiation 21 s.

Key:

1. Mating periods, weeks
2. Effect variant
3. Number of implanted
4. Percent of effective crossings
5. Yellow bodies per
6. Places of implantation per
7. Live embryos per
8. Survival rate, percent
9. Mortality
10. Before implantation, percent
11. After implantation, percent
12. First
13. Super high frequency
14. Control
15. Second
16. Third
17. Fourth
18. Fifth
19. Sixth
20. Total

Table 2. Embryonal Mortality in the Offspring of Males Subjected to a Fractionated Action of SHF Microwaves

(1) Срок спаривания, недели	(2) Вариант опыта	(3) Генотип ♀	(4) Количество подсаженных ♀	(5) Проц. эффективных скрещиваний	(6) Желтых тел на ♀	(7) Мест имплантации на ♀	(8) Живых эмбрионов на ♀	(9) Проц. выживаемости	Смертность (10) до имплантации (11)	Смертность (10) после имплантации (12)
									Проц. (13)	
(14) 1-я	(15) СВЧ	(16) Б/п	215	46,5	10,2	8,9	7,7	76,3	12,0	13,3
	(17) Контроль		159	34,6	10,3	9,0	8,1	78,9	12,1	10,3
	(15) СВЧ	Гибриды (18)	132	77,3	10,7	9,6	8,9	83,2	10,6	6,9
	(17) Контроль		117	80,3	10,5	9,4	9,0	85,7	9,8	5,0
(19) 2-я	(15) СВЧ	(16) Б/п	215	56,7	10,6	9,4	8,3	78,1	13,8	9,4
	(15) Контроль (17)		159	69,8	9,9	8,9	8,1	81,9	10,8	8,2
	(15) СВЧ	Гибриды	135	81,5	10,6	9,3	8,8	82,5	12,2	6,0
	(17) Контроль	(18)	117	82,0	10,5	9,1	8,8	83,5	13,4	3,7
(20) 3-я	(15) СВЧ	Б/п	114	71,9	10,8	9,5	8,8	81,3	11,9	7,7
	(17) Контроль	(16)	99	73,7	10,5	9,8	8,9	84,7	7,0	9,0
(21) 4-я	(15) СВЧ	Б/п	117	65,8	11,5	10,4	9,5	82,5	10,0	8,4
	(17) Контроль		99	68,7	12,3	10,7	9,0	72,9	13,4	15,8
(22) 5-я	(15) СВЧ	Б/п	106	79,2	11,9	10,1	9,3	78,2	14,8	8,1
	(17) Контроль	(16)	89	73,0	12,0	10,1	8,9	74,5	16,2	11,2
(23) Всего	СВЧ (15)		1034	65,5	10,8	9,5	8,7	80,2	12,3	8,5
	(17) Контроль		839	67,0	10,8	9,5	8,7	80,7	11,8	8,5

Key:

1. Mating period, weeks
2. Experimental variant
3. Genotype
4. Number of implanted
5. Percent of effective crossings
6. Yellow bodies per
7. Places of implantation per
8. Live embryos per
9. Percent of survival rate
10. Mortality
11. Before implantation
12. After implantation
13. Percent
14. First
15. Super high frequency
16. Sterile
17. Control
18. Hybrids
19. Second
20. Third
21. Fourth
22. Fifth
23. Total

The results of study of the effect of SHF microwaves on bone marrow cells are presented in table 3. As follows from the data, the percent of aberrant metaphases in the experiment and control comprised the same values.

Thus, not a single test used by us disclosed a mutagenic effect of SHF microwaves under the given experimental conditions. A comparison of our results with the data on this problem in the literature often is difficult, because the conditions

of irradiation are not comparable. Thus, with a local irradiation of mouse testes a weak increase in the frequency of induced dominant lethal mutations was obtained /6, 7/. However, no pattern in a rise in the level of mutations depending on the stage of spermatogenesis subjected to the effect was observed. If another method of statistical processing is used, that is, the number of yellow bodies, places of implantation and live embryos is presented in the form of an average number per female, the differences between the experiment and control in these studies will prove to be insignificant. Another author /5/ obtained a mutagenic effect of SHF microwaves under a fractionated action on bone marrow cells. However, the conditions of irradiation in our experiment differ from those used in the above-mentioned study, which makes a comparison of results difficult. An analysis of the data from sources in the literature available to us makes it possible to assume that, apparently, the degree of a mutagenic effect or its absence depends primarily on the conditions of the action. In the opinion of some authors, to this day there are no direct proofs of a specific nonthermal effect of SHF microwaves /17/, because there are no data on the existence of a molecular interaction of the biosystem with the absorbed energy of a SHF electromagnetic field. Proceeding from the above-stated, it is possible to explain the lack of a mutagenic effect in our experiments.

Table 3. Frequency of Chromosome Aberrations in Bone Marrow Cells of Mice as a Result of a Fractionated Irradiation With SHF Range Microwaves

Action	Number of Examined Metaphases		Percent of Aberrant Metaphases
	Total	Aberrant	
SHF	740	12	1.62
Control	372	5	1.37

When this article had already been prepared for the press, a study confirming the data obtained by us on the lack of a mutagenic effect of SHF microwaves in sex cells of mammals appeared. The authors, varying the time of a chronic irradiation of male rats with SHF microwaves with energy flux density of 5 and 10 mW/cm^2, did not detect induced dominant lethal mutations or a decrease in the fertility of animals /18/.

Conclusions. As a result of the study of a single action of SHF microwaves with energy flux density of 60 and 800 mW/cm^2 and of a fractionated action of SHF microwaves with energy flux density of 800 mW/cm^2 on sex and somatic cells of male mice, a mutagenic effect of this factor was not revealed by any of the three used tests (frequency of induced dominant lethal mutations; frequency of anomalous spermium heads and frequency of chromosome aberrations in bone marrow cells).

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IMMUNOMORPHOLOGICAL CHANGES IN THE TESTES UNDER INFLUENCE OF SUPERHIGH-FREQUENCY ELECTROMAGNETIC FIELD

Leningrad ARKHIV ANATOMII, GISTOLOGII I EMBRIOLOGII in Russian Vol 80, No 2, Feb 81 pp 69-75

[Article by V. V. Grigor'yev, R. P. Ogurtsov and Yu. N. Zubzhitskiy, Department of Histology and Embryology (headed by Prof A. A. Klishov), Military Medical Academy imeni S. M. Kirov; Department of Microbiology and Immunology (headed by Prof B. N. Sofronov), Scientific Research Institute of Experimental Medicine, USSR Academy of Medical Sciences; Scientific Research Laboratory of Electron Microscopy and Histochemistry (headed by K. K. Zaytseva, doctor of medical sciences), Military Medical Academy imeni S. M. Kirov, submitted 14 Feb 80]

[Text] The reactions of organs isolated from the effects of immunological factors by histohematic barriers are of special interest to experimental morphology. It is possible to investigate the role of antibodies and sensitized lymphoid cells in development of autoimmune processes on models of damage to the brain, peripheral nervous system, testes, thyroid and eye (V. V. Serov, 1968; A. D. Ado, 1972; A. I. Strukov, 1972).

Heretofore, the study of the testes by immunomorphological methods had been conducted in experiments where traumatic factors (S. S. Raytsina, 1970), immunization with antigens of homologous testes (J. Freund et al., 1955: B. H. Waksman, 1959; P. C. Brown et al., 1963) and cross-reacting antigens of microorganisms (R. P. Ogurtsov and Yu. N. Zubzhitskiy, 1978) were the triggering mechanisms of the process.

A superhigh-frequency electromagnetic field (SHF EMF) of nonthermal intensity elicits aspermatogenesis and development of lymphoid infiltrates in testicular interstitial tissue, even with brief exposure (V. V. Grigor'yev, 1978). Since this effect cannot be attributed to the thermal effect of the field on cells, there is reason to believe that the damage is possibly related to lesion to the hematotesticular barrier (HTB) and is associated with development of an immunopathological process.

Our objective here was to make a study of the possibility of development of an immune reaction to testicular antigens and its relation to impairment of structure and permeability of the HTB under the influence of SHF EMF of nonthermal intensity, using immunomorphological, histological methods and electron microscopy.

Material and Methods

Experiments were conducted on 77 puberal male rabbits weighing 2.5-3 kg. The testes were submitted to local irradiation for 6 min delivered from a Luch-58

radiotherapy machine (wavelength 12.6 cm, intensity 8.8 mW/cm^2). The control group consisted of 15 nonirradiated rabbits. The animals were weighed 1, 3, 5, 7, 14, 21, 30, 60, 90 and 210 days after exposure, sacrificed by decapitation, after which the testes, blood and spleen were taken for examination. We determined the ratio of red to white pulp mass of the spleen by the method of Gammar, as well as weight of the testes and the animal. Various parts of the same testis were fixed according to Carnoy and Colfield, the spleen according to Carnoy and imbedded in paraffin, and the testes were also imbedded in epon 812. Part of the testis was frozen in a mixture of ice and petroleum ether to obtain cryostat sections. The paraffin sections were stained with hematoxylin-eosin, while the ultrafine sections were contrasted with uranyl acetate and lead nitrate. The preparations were viewed under a light microscope and type JEM 100 CX electron microscope.

For demonstration of antibody globulin fixed in the testes, the cryostat sections were eluated for 10 min in phosphate buffer (pH 7.2) and, after drying, incubated with FITTs [fluorescein isothiocyanate]-labeled antirabbit serum (produced by the Scientific Research Institute of Epidemiology and Microbiology imeni N. F. Gamaleya, USSR Academy of Medical Sciences). Antibodies to antigens of rabbit testes were assayed in blood serum with the complement fixation test under refrigeration (V. I. Ioffe and K. M. Rozental', 1943) with homologous testicular extract in a dosage of 1 mg/mℓ protein and by the indirect immunofluorescence method (T. H. Weller and A. H. Coons, 1954), using cryostat sections of homologous testes fixed in 96° ethanol as antigen.

Hypersensitivity of the delayed type was tested after 9, 14, 30 and 90 days, for which purpose a group of 17 experimental and 6 control animals was given intracutaneous injections of homologous testicular extract (in doses of 1-0.01 mg/mℓ per rabbit). The reaction was evaluated 24 h after the injection.

In order to examine permeability of the HTB 1 and 14 days after irradiation, we gave intravenous injections of 3 mℓ ox serum albumin in a concentration of 100 mg/mℓ/kg animal weight, conjugated with rhodamine chlorideor Evans blue dye. After 2 h, the testes were removed, cryostat sections prepared and examined under an ML-4 fluorescence microscope with UFS-3 and SZS-14 light filters, and ZhS-3 ocular filter.

Results and Discussion

The experimental animals were divided into three groups, according to the individual reactions to the deleterious effects of SHF EMF.

The first group (25 rabbits) developed mild reactive changes in the testes, manifested by death of only a few cells of the spermatogenic epithelium in both centrally situated tubules and near the testicular membrane after 1-3 days. After 5-6 days, spermatogenesis was virtually normal.

Already 1 day after exposure, the second group (22 rabbits) presented microscopic hemorrhages, which were the most extensive in the region of the testicular rete. Lymphoid infiltrates were formed in areas with hemorrhages after 2-5 days. The damage to the testis was notably nonuniform: complete atrophy of spermatogenic epithelium in the region of hemorrhages and adjacent zone, confined to the lobules, whereas in tubules of adjacent lobules removed from the lesion site the process of spermatogenesis continued.

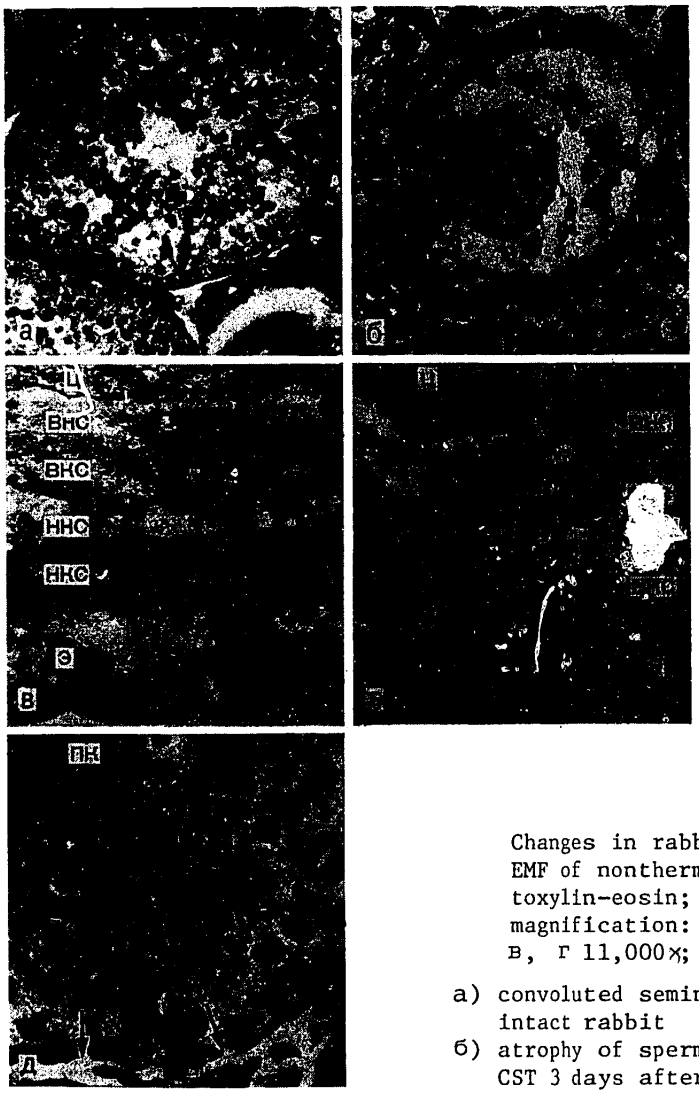

Figure 1.
Changes in rabbit testes exposed to SHF EMF of nonthermal intensity (а, б hematoxylin-eosin; д fluorescence microscopy; magnification: а, б obj. 20×, ocul. 10×; в, г 11,000×; д obj. 40×, ocul. 7×)

а) convoluted seminiferous tubules [CST] of intact rabbit
б) atrophy of spermatogenic epithelium of CST 3 days after SHF EMF; arrow points to lymphoid infiltrate (in intertubular connective tissue
в) tunica propria of CST of intact rabbit
ВНС) internal noncellular layer
ВКС) internal cellular layer
ННС) external noncellular layer
НКС) external cellular layer
Ц) sustentocyte cytoplasm
Э) capillary endothelium
ПК) tubular lumen
г) tunica propria of CST of experimental rabbit 14 days after SHF EMF; designations are the same as in в
д) accumulation of rhodamine chloride labeled ox serum albumin in intertubular connective tissue and CST 14 days after SHF EMF

Dynamics of demonstration of delayed type reactions to homologous testicular antigens after exposing rabbits to SHF EMF

Experimental conditions		Reaction parameters	Dose of injected testicular extract (mg)		
Postradiation testing time (day)	Total animals		1,0	0,1	0,01
7	3	Number of animals with positive reactions	2	1	1
		Mean diameter of erythema (mm)	8,5	6,0	8,0
14	3	Animals with positive reactions	3	3	1
		Mean diameter of erythema (mm)	17,0	13,0	12,0
30	6	Animals with positive reactions	5	3	1
		Mean diameter of erythema	9,2	8,0	9,0
90	2	Animals with positive reactions	2	2	2
		Mean diameter of erythema	32	31,5	24,5
210	3	Animals with positive reactions	0	0	0
		Mean diameter of erythema	—	—	—

Note: Different doses were given repeatedly to the same animal.

In the third group (30 animals), the changes in spermatogenic epithelium were the most significant; after 1 day spermatozoa disappeared, spermatids and spermatocytes were sloughed off, the number of multinuclear elements derived from sex cells increased. After 2-3 days, there was appearance of lymphoid infiltrates (chiefly in interstitial tissue near the tunica albuginea) (Figure 1, а, б). Some lymphoid cells infiltrated the seminiferous tubules. At the later postradiation stages, the dystrophic changes in convoluted seminiferous tubules and lymphoid infiltration of testicular tissues progressively increased and reached a maximum between the 6th and 14th day.

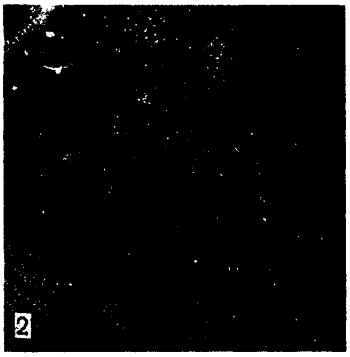

Figure 2.
Bright fluorescence of spermatids in testes of intact rabbit, which were treated with experimental rabbit serum taken 20 days after exposure to SHF EMF of nonthermal intensity; indirect immunofluorescence method; obj. 40×, ocul. 7×

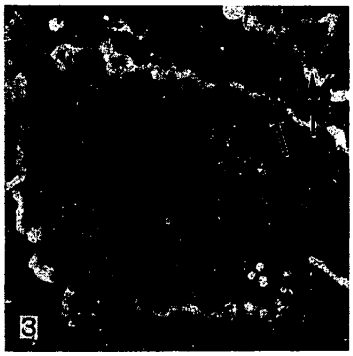

Figure 3.
Fixing of globulins on tunics of convoluted tubules of rabbit testis 90 days after exposure to SHF EMF of nonthermal intensity; arrows point to fixation sites; direct fluorescence method; objective 40×, ocular 7×

After 14 days, electron microscopy revealed severe changes, primarily in the structure of the tunica propria of the convoluted tubules (see Figure 1, в, г). There was no longer parallel arrangement and spatial correlation between layers; there was an increase in amount of vesicular structures in them, as well as in the cells of the basal zone of the tubules, particularly in sustentocytes. The cells no longer had a capacity for pinocytosis, whereas their topography became jagged due to intensified flow of metabolic products through them.

Testicular atrophy was associated with reduction of their mass in the second and third groups of animals. The relative mass of the testes decreased to $90 \cdot 10^{-5} \pm 7 \cdot 10^{-5}$ after 3 days, remaining low after 5 days and coming close to normal ($140 \cdot 10^{-5} \pm 25 \cdot 10^{-5}$) only after 7 days. The third group of animals presented loss of mass again after 60 and particularly after 90 days (to $95 \cdot 10^{-5} \pm 3 \cdot 10^{-5}$).

The lesion demonstrable at the early stages could be related to the deleterious effects of SHF EMF on testicular tissues. Appearance of lymphoid infiltration at the later stages was indicative of development of an immune reaction to testicular antigens (B. H. Waksman, 1959; S. S. Raytsina, 1970). This reaction could be directed to antigens of both injured and uninjured tissue, if they entered the blood stream due to impairment of HTB structure after exposure to the field. To test this assumption, experiments were conducted to evaluate permeability of the HTB under the influence of SHF EMF.

Already 1 day after exposure, there was accumulation of albumin in interstitial tissue between the seminiferous tubules in the region of the vessels, and after 14 days albumin was demonstrable within the tubules (see Figure 1, д), which was apparently related to impaired permeability of the HTB.

Antigenic stimulation of lymphoid tissue altered the proportion of white and red pulp mass of the spleen. It constituted 0.16 in control rabbits, 0.23 in experimental animals after 1 day, with reduction of white pulp mass to almost one-half after 3 days. Later on, the mass thereof increased again, reaching a maximum after 14 days (0.34), again reverting to normal after 21 days; however, it increased reliably again after 30 days.

In some rabbits, complement-fixing antibodies (in a titer not exceeding 1:10) appeared in blood serum 5-7 days after exposure to SHF field. Starting on the 20th day, they were demonstrable only in animals of the second and third groups, in titers of 1:20-1:40. The blood serum of these groups of rabbits also presented antibodies on the 20th day (in titers of 1:20-1:40) capable of specific reactions in the indirect immunofluorescence method with spermatid structures on sections of the testis (Figure 2). Examination of cryostat sections of testes treated with fluorescein isothiocyanate labeled antirabbit serum revealed fixing of globulins both on spermatids and spermatozoa, as well as the external surface of the tunica propria of the seminiferous tubules, 20, 30 and 90 days after exposure to SHF EMF (Figure 3). Complement-fixing and fluorescent antibodies were demonstrable up to the 90th day after exposure (Figure 4), whereas in the third group of animals complement-fixing antibodies were even demonstrable after 7 months, in a titer of 1:20.

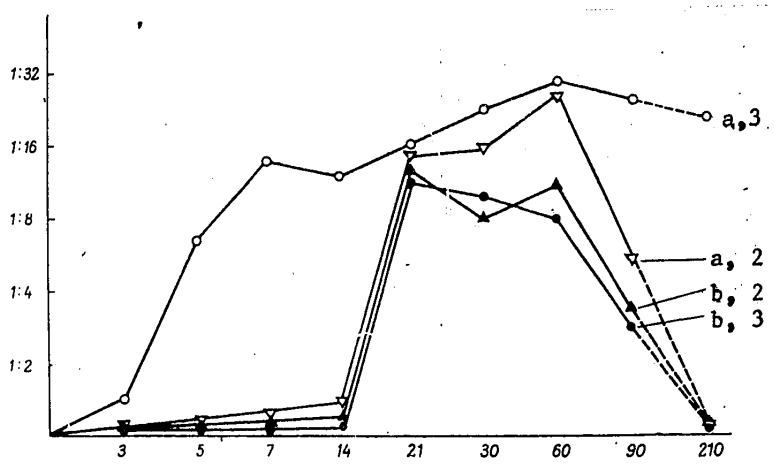

Figure 4. Dynamics of demonstration of antibodies to testicular antigens in rabbits after exposure to SHF EMF of nonthermal intensity. X-axis, postradiation time (days); y-axis, antibody titer

a) complement-fixing antibodies 2, 3) animal groups
b) fluorescent antibodies

The reaction of hypersensitivity of the delayed type revealed positive skin tests in the second and third groups of animals after 14 days; however, they were demonstrable after 30 and 90 days, with build-up of intensity, only in the third group (Table).

Conclusion

The capacity of blood serum antibodies to react in the indirect fluorescence test with spermatid structures is indicative of their being directed against autoantigens of the testis (G. A. Voisin and F. Toullet, 1969).

There are grounds to believe that the immunological reaction complicates the course of the reactive process in the testis. This is related, in the first place, to development of hypersensitivity of the delayed type to autoantigens and appearance of lymphocytes in testicular tissue and, in the second place, to appearance of antibodies capable of fixing complement and having a cytotoxic effect on testicular cells. In the latter case, the impaired permeability of the HTB allows immunoglobulins to enter into the convoluted seminiferous tubules or interact with autoantigens discharged from the seminiferous tubules. Different antigens are fixed differently on testicular structures. The two types of fixation of globulins that we demonstrated (on the spermatogenic epithelium and tunica propria of the tubules) confirm this assumption.

Thus, local exposure of the testes to superhigh-frequency electromagnetic fields of nonthermal intensity shows that both immunological mechanisms--humoral (antibodies) and cellular (sensitized lymphocytes)--have a deleterious effect on the testes in the presence of impaired HTB permeability.

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EFFECT OF INDUSTRIAL CURRENT ELECTROMAGNETIC FIELD UPON NATURE OF GROWTH AND MITOTIC ACTIVITY OF HUMAN FIBROBLAST CELL CULTURES

Kiev TSITOLOGIYA I GENETIKA in Russian Vol 15, No 3, May-Jun 81 (manuscript received 3 Dec 79) pp 9-12

[Article by V.D. Dyshlovoy, A.S. Panchuk, and V.S. Kachura, Kiev Medical Institute]

[Text] Introduction. Growing interest in the study of the effects of physical factors of an electromagnetic nature upon man and the environment has come about as the result of widespread introduction of generating sources for those factors. Specifically, a need for researching the nature of changes occurring in animals and man resulting from the effects of electromagnetic fields of 50Hz (EMF 50Hz) has arisen in conjunction with the introduction into operation of electric power transmission lines (LEP) of the high and super-high current categories having powerful electromagnetic fields in the area of the conducting surfaces [1,2]. Although data on the nature of biological effects and the degree of potential danger are at this time questionable, there is no doubt that it is becoming an important ecological and hygienic factor [3-5]. Additionally, the study of the nature of changes occurring in the organism and its cells as the result of the effects of electromagnetic fields is one of the most powerful instruments for learning their structures and functions, as many processes in the organism are electromagnetic transformation based.

Particularly significant in establishing the nature and biological effect of EMF, specifically its direct effect upon cellular structure would be research employing cell cultures as a model subject [6]. This article presents study results relating to the nature of changes occuring under the influence of 50Hz EMF in human embryonic cell cultures.

Materials and Methods. Subject of the research was primary cultures of embryonic fibroblast cell cultures from human embryos, cultivated in covered glass according to accepted methods. The electromagnetic field was created between plates of a capacitor in a thermostatically controlled chamber. Required field current was achieved by varying the current on the plates or the distance between them. After irradiation, the preparations were secured, air-dried, stained with acetoorcein or Meyer's hematoxylin with eosin dye. The mitotic index was established using the MBI-3 microscope with an estimated 5000 cells and expressed per thousand. Statistical analysis of digital material was made with the "Mir-1" computer employing the Student-Fisher method for comparing aggregates with mutually related variants. The studies were conducted using 58 cultures.

Research results and discussion. Under normal conditions, human embryonic fibroblast cells in culture are polygonal in shape, and possess a large number of cytoplasmic extensions or appendages, which, as a rule, contact the extensions of other cells, forming at given stages of culture development the so-called "monolayer". Layers of culture monolayer cells have a different orientation in the substrate. With daily 5-hour exposure to 50Hz EMF, on day 5 or 6, a reduced number of cytoplasmic extensions is noted in the fibroblast cells. On subsequent days, the cells are more rounded, and often lose contact with each other. With constant 24-hour exposure to field effect of the same or different current, changes are analogous, but somewhat more expressed during the same study periods. Subsequently, a portion of the cells creeps from the substrate, and "windows", free from cells, appear on the glass, usually colonized by young cells. In those cultures subjected to EMF exposure, morphological characteristics of the nuclear segments of the cells change, that is, the number of improperly formed nuclei--dense, vacuolized, and containing large quantities of condensed chromatin, increases.

Table 1

Change of Mitotic Index in Cultured Fibroblast Cells of Human Embryo Exposed to 50Hz EMF 50kV/m Current

Exposure time to EMF, hours	Mitotic Index, o/oo		n	t	P
	Control	Experiment			
0.5	5.4+0.4	5.8+0.5	10	1.9	less than 0.05
3.0	6.2+0.7	5.8+0.5	10	0.5	less than 0.1
6.0	4.8+0.8	3.8+0.7	10	0.8	less than 0.1
12.0	4.9+1.1.	3.9+0.4	10	0.8	less than 0.1
24.0	5.0+0.4	3.4+0.2	10	5.1	more than 0.001

With intensified field current to 100kV/m, rounding of the cytoplasm was recorded by day 3 or 4, and subsequently a segment of cells loses contact with each other, and the orientation of cell strata in the monolayer is disrupted. The effect of a 150kV/m field results in more clearly expressed degenerative changes: the cytoplasm concentrates, cells reduce in dimension, frequently lying isolated, monolayer strata orientation is disrupted, and nuclei virtually devoid of cytoplasm are encountered. With extended exposure to EMF, nuclear forms change, forming "windows" as the result of a segment of cells creeping from the substrate; those "windows" are not colonized by young cells. At the same time, total loss of irradiated cultures was observed only in single instances. It must be noted, that rapidity of onset and pronounced nature of changes were dissimilar in various cultures, which apparently is related to the condition of the initial embryonic material from which the cultures were prepared, and to individual peculiarities of their development.

Parallel with an evaluation of the general state of the cultures in a dynamic state with 1 to 8 day exposure of EMF 50kV/m current, certain indicators were studied relating to the rate of their reproduction by determining mitotic activity.

Research demonstrated that under normal conditions, the number of cells dividing in the cultures throughout the entire period of observation was maintained at the same approximate level. The average value of the mitotic index was 1.4--10.20/00, characteristic for cultures in the stationary development period. Under the effect of the field, a gradual reduction in the number of dividing cells is seen and, toward the end of the 24-hour period of exposure to EMF, differences attained statistically significant levels (table 1). With increased exposure time, the mitotic index drops by an approximately equal value in every observation period.

The results obtained in this series of experiments provide a basis for hypothesizing that increases in exposure times to the field will result in a more pronounced reduction in the number of dividing cells. To verify this hypothesis, the following series of experiments were conducted, in which the time of field exposure was increased to 48 hours with the same intensity.

Table 2

Changes of Mitotic Index in Human Embryo Fibroblast Cell Cultures After 48-hour Exposure to 50Hz EMF of 50kV/m Intensity

Time of Observation After Cessation of Exposure to EMF, hours	Mitotic Index, 0/00		n	t	P
	Control	Experiment			
0.0	7.8+0.8	4.8+0.6	8	3.0	less than 0.01
3.0	7.8+0.7	5.0+0.8	8	3.1	less than 0.01
6.0	7.6+0.9	4.6+0.7	8	2.6	less than 0.05
12.0	7.6+0.5	5.8+0.9	8	1.7	more than 0.05
24.0	7.8+0.7	6.0+0.8	8	1.6	more than 0.05

Table 3

Change of Mitotic Index in Cultured Human Embryo Fibroblast Cells After Differing Periods From Onset of Exposure to 50Hz 50kV/m Intensity EMF

Exposure Time to EMF, hours	Mitotic Index, 0/00		n	t	P
	Control	Experiment			
48.0	10.2+0.68	7.2+0.6	10	3.0	less than 0.01
96.0	2.0+0.4	4.0+0.6	10	8.7	less than 0.001
120.0	3.2+0.8	5.2+0.6	10	3.0	less than 0.01
144.0	1.4+0.3	3.4+0.4	9	3.6	less than 0.01
168.0	2.2+0.8	3.6+0.4	9	2.9	less than 0.05

As can be seen from table 2, after a 48-hour field exposure during the experiment, a statistically significant (P less than 0.01) reduction in the mitotic index of 29.7% is noted. Subsequently, after cessation of the exposure, mitotic activity in the experimental testtubes grows more intensively than in the control, and in 12 hours, the differences between the control and the experiment become statistically insignificant (P greater than 0.05).

Comparison of the results from the first two series of experiments indicates that with increased time of field exposure by a factor of two, no adequate increase of reaction in cultures including increased degree of decreased mitotic activity was observed. This may be related to the fact that the researched factor, after a short-term exposure is sufficient to disrupt the normal course of the cellular cycle, and further increases in time of its effect does not lead to more pronounced changes. It can also be hypothesized that the reduction in the mitotic index occurs as the result of damage to only a part of the cells, i.e., those most sensitive at a particular time to the effect of EMF. A second, more resistant group of cells continues to pass through the cellular cycle and divide.

To verify the hypothesis regarding the absence of a direct link between extended field exposure and the degree of reduction in the mitotic index, the following series of experiments exposed cultures to a field over a 7-day period. Every 1-2 days, the mitotic index of a segment of testtubes in the control and the experiment was determined.

As in the previous series of experiments, after a 48-hour irradiation, a statistically significant (P less than 0.001--0.05) reduction of the mitotic index was noted (table 3). However, after 96 hours of exposure to EMF, the effect was the opposite--the number of dividing cells in the experiment exceeded by twofold the number of such cells in the control. With further irradiation, differences between the control and the experiment grew, and only after a 7-day exposure did the intensity of the growth in the mitotic activity in them begin to drop.

This may be related to the fact that after 5-6 days of exposure to the cultures, contact retardation is reduced as the result of losing a segment of the cells. The result of this is a growth in mitotic activity, as in initially trypsinized human embryo fibroblast cell cultures in the stationary development period, there is always present a significant number of cells in the C_2 period. The hypothesis that the increase in the number of mitotic figures with extended field exposure is tied to the retarded course of the cell division process itself, appears unlikely, as the field energy is far too low to disrupt the mitotic process.

Conclusions. Exposure of human cell cultures to 50Hz EMF results in disruptions to growth and development, which may be evaluated as evidence of the direct effect of this factor upon cellular structures and processes. A certain parallelism is observed between the intensity of the field and the time of exposure on the one hand, and the pronounced character of culture morphological changes and their mitotic activity on the other. It is most likely that the changes manifested in the condition of the cultures under the effect of 50Hz EMF are related to the suppression of the synthetic processes in the cells.

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ACUTE EXPERIMENTAL EMOTIONAL STRESS IN RABBITS (PHYSIOLOGIC-CYTOCHEMICAL ASPECTS)

Moscow IZVESTIYA AKADEMII NAUK SSSR: SERIYA BIOLOGICHESKAYA in Russian No 1, Jan-Feb 81 (manuscript received 11 Apr 80) pp 45-52

[Article by A.V. Gorbunova, N.V. Petrova, V.V. Portugalov and S.K. Sudakov, Institute of Normal Physiology imeni P.K. Anokhin, USSR Academy of Medical Sciences, Moscow]

[Text] Comprehensive physiological-cytological studies have determined the effect of acute experimental emotional stress created by electrical stimulation of the hypothalamus and skin in immobilized mature male rabbits of the chinchilla variety.

Three groups of animals were isolated according to the nature of their cardio-vascular reactions: stable, adaptive, and predisposed to the development of stress. In those animals predisposed to the development of emotional stress, there were indications of dissimilar metabolic shifts in various extramural ganglia of the autonomic nervous system and the sympathetic network. The most pronounced changes occur in the nodal ganglion of the vagus nerve; here changes of a catabolic nature predominate over anabolic. In the stellate ganglion and sympathetic network nodes, an intensification of anabolic processes occurs. The superior cervical sympathetic node by virtue of the nature of metabolic changes occupies an intermediate position. The conductive system of the heart demonstrates a tendency toward increased activity of the rapid isoenzymes of lactate dehydrogenase.

In recent years, it has been firmly established that in acute experimental emotional stress, vascular disruptions of varying manifestations occur (Bakulin and coauthors, 1976; Dashzeveg, 1973; Sudakov, 1972). The research of Ye.A. Yumatov and Yu.G. Skotselyas (1979) determined individual peculiarities of cardiovascular disruptions or irregularities in rabbits of differing genetic lines with emotional stress. This work undertakes attempts to isolate individual peculiarities of the dynamics of cardio-vascular reactions in rabbits manifesting acute experimental emotional stress.

Although paramount significance is attributed to the peripheral autonomic nervous system in the development of stress reactions, there is a complete absence of data in literature which characterizes the degree of involvement in these reactions of its separate cellular formations under the effect of stress generating factors

upon the animals' organisms, much loss upon animals with varying stability of cardio-vascular functions to the effects of such a phenomenom. The latter compels us to focus attention on the metabolism and condition of neurons in the extramural nodes of the autonomic nervous system, anatomically related to the heart.

Materials and Methods

The research was conducted on 49 male rabbits of the chinchilla variety weighing 2.5 kg which had electrodes for stimulation implanted beforehand in the area of the ventro-medial nuclei of the hypothalamus. Only those animals displaying a passive-defensive reaction to the electrical current stimulation of the ventro-medial nuclei of the hypothalamus (intensity 2-8 v, 50Hz frequency) were selected for the experiment.

During the experiments, the rabbits were kept immobilized in stalls, and administered alternating and simultaneous short-term stimulation to the hypothalamus and various skin segments. Aperiodic stimulation demonstrated the most pronounced stressogenic effects (Abramov, 1973; Mamedov, Shumilina, 1975; Weiss, 1971). The electrical stimulation was effected using needle electrodes implanted in the skin of the front and rear extremities, and also by special clamps or terminals attached to the rabbits' ears. Imputs were employed which elicited an elevation of arterial pressure on the order of 20-30 mm of mercury without a pronounced motor reaction of the animal. The rabbit received six stimulations during a 10 minute period. An impulse current 10-15v, 50Hz was employed for nociseptive effect. Duration of a single impulse was 20 msec. Each stimulation lasted from 1-10 secs. Each animal had a catheter implanted in the femoral arterty, and through the use of a tensometer and myngograph of the "Siemens-Elema" firm, the arterial pressure and cardiac contraction frequency were recorded.

The condition of the neurons and the metabolism of certain cellular formations of the peripheral autonomic nervous system was studied using cytochemical methods providing information on indicators noticeably changing during the process of the nervous system functioning. Thus, the results of the biochemical and cytochemical research uniformly demonstrate the presence of relationships between the state of functional activity and the level of nerve tissue protein metabolism (Palladin, 1959; Brodskiy, 1966). Based upon the example of the study of proteins differing in chemical properties in structures of the nodal, superior cervical, and stellate ganglia, to include the segment of the sympathetic circuit at a level of 4-6 thoracic segments, an attempt was made to determine the nature of changes occurring in them during the course of acute emotional stress. Changes in metabolism of nerve cells also served as an indicator of their functional state (Geynisman, 1974). The content of water soluble proteins in the structures studied was determined according to Lowry (Lowry, et al., 1951). The results were processed statistically according to the non-parametric criterion of Van der Varden (1960). To establish the dry weight of the cytoplasm and neuron nuclei, volumes of nuclei and nerve cell bodies of the nodal-vagus nerve and the superior cervical sympathetic ganglion were fixed in Carnot's liquid and poured into paraffin. The dry weight of the cytoplasm and neuron nuclei was determined by the interferometry method with sections 7mcm using an interferometric microscope of the "Opton" firm. The volume of neurons studied and their nuclei was determined according to the formula of ellipsoidal rotation

$$v = \frac{\pi D d^2}{6} .$$

Research of mononuclear and dinuclear cells of the superior cervical ganglion was conducted separately. All digital material was processed statistically according to the method of variational statistics (Plokhinskiy, 1970). The correlation of aerobic and anerobic carbohydrate conversions was studied using the electrophoresis method in polyacrylamide gel, determining content of lactate dehydrogenase (LDG) isoenzymes in the cited formations of the nervous system as well as in the functioning organ--the heart, anatomically related to these structures, and separately in the conductive system of the heart, isolating the atrioventricular fascicle with elements of the right crus of the His bundle (A-V bundle) (Umovist, Sinev, 1969). A quantitative evaluation was made of the activity of individual isoenzymes using the recording IFO-451 microphotometer produced by the Kazan' Optico-Mechanical Plant.

Serving as control in the cytochemical research were rabbits maintained in a vivarium.

According to the nature of changes in arterial pressure in animals under emotional stress, it was possible to isolate three groups irrespective of initial hemodynamic parameters (fig. 1).

The first group was composed of rabbits whose arterial pressure did not change throughout the experiment. An instability of frequency of cardiac contractions was seen; an increase in it and a decrease, as well, was noted. Animals of this group (4 rabbits) we designated as stable to the action of stressogenic factors.

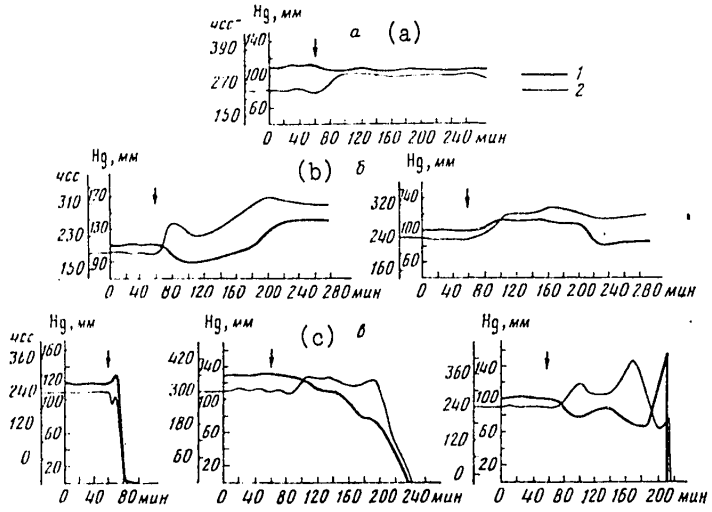

Figure 1. Nature of Cardio-Vascular Reactions in Rabbits of Varying Groups: a--stable, b--adaptive, c--predisposed. X-axis depicts time of experiment in minutes, and Y-axis--frequency of cardiac contractions per minute and value of arterial pressure in mm of mercury. The arrow indicates onset of exposure; 1--arterial pressure, 2--frequency of cardiac contractions (ChSS)

The second group of animals (11 rabbits) developed changes in arterial pressure not resulting in death. With a portion of the rabbits, arterial pressure at the beginning of the experiment dropped by 17.0±11.3 mm of mercury, and frequency of cardiac contractions increased by 50 in 1 minute. A gradual increase of arterial pressure and a certain reduction of cardiac contraction frequency was then observed. Within 1.5--2.0 hours after the beginning of the experiment, arterial pressure increased by 28.0±20.6 mm compared to the initial level, and the frequency of cardiac contractions on average increased by 90 in 1 minute (5 rabbits). The other set of animals (6 rabbits) demonstrated increased arterial pressure at the onset of the experiment by 5.8±3.2 mm, and within 1.5--2.0 hours a steady reduction of 23.3±2.7 mm from the initial level. Frequency of cardiac contractions for the duration of the experiment increased by 50--80 per 1 minute. The animals of this group we evaluated as adaptive.

The third group included animals termed predisposed to the development of stress, dying during the course of the experiment (34 rabbits). This group was broken down into three subgroups. The first subgroup included those animals (8 in number) which expired within 15--40 minutes from the beginning of the experiment. Those animals evidenced, with the administering of the initial stimulations, elevation of arterial pressure by 16.0±9.4 mm with insignificant increase or reduction in cardiac contractions, followed by a sharp reduction of arterial pressure, cardiac contraction frequency, and death. Rabbits of the second subgroup (16 animals) with the onset of the experiment demonstrated a gradual drop in pressure, resulting in their deaths within 1.5 to 2.0 hours. Frequency of cardiac contractions in these rabbits for the duration of the experiment was, on the average, 55 higher than the initial level and reduced to the moment of death. With animals of the third subgroup (10 rabbits), a unique phasic nature of changes was detected. With onset of the stimulation, the arterial pressure either increased insignificantly, or did not change; frequency of cardiac contractions remained stable. After this, arterial pressure dropped below the initial level by 13.0±4.7 mm, and remained so for 1.0 to 1.5 hours with a frequency of cardiac contractions 50-100 per 1 minute greater than the initial frequency. A subsequent secondary elevation of arterial pressure then developed by 40.0±11.3 mm, accompanied by a reduction in cardiac contraction frequency to the initial level. Death occurred at the peak of the arterial pressure elevation.

Thus, data was obtained indicating that in a population of rabbits of the chinchilla variety, individual animals are encountered demonstrating varying stabilities of cardiac-vascular functions to acute experimental emotional stress.

Cytochemical research has demonstrated that a definite reduction of watersoluble protein content occurs in nodal ganglia homogenates in rabbits predisposed to the development of stress (Table 1). In homogenates of the stellate ganglion, a definite increase in their content was detected relative to the control, and in the sympathetic circuit, only a tendency toward increased content was noted. The content of watersoluble proteins in homogenates of the superior cervical ganglion did not differ from the control animals.

Table 1

Content of Watersoluble Proteins in Extramural Ganglia of the Autonomic Nervous System Under Stress
mkg/mg of untreated tissue

Research subject	Control	Stress	X_1
Superior cervical ganglion	48	48	
Nodal Ganglion	45	36*	3.63
Stellate ganglion	43	50*	3.29
Sympathetic circuit	33	41	2.12
Number of animals	12	7	
X_0			3.24

X_1 and X_0 -- conventional units, calculated table values with level of significance 5%

* In tables 1-6--reliable difference as compared with control

A morphological analysis indicated that in those rabbits predisposed to the development of stress, the volume of cell bodies with mononuclear neurons of the superior cervical and nodal ganglia and their nuclei, as well as the volume of dinuclear neurons of the superior cervical ganglion was definitely reduced relative to the control (tables 2,3). The weight of dry cytoplasm and nuclei of mononuclear neurons, nuclei of dinuclear neurons of the superior cervical ganglion, and of nodal ganglion nuclei were substantially below the level of the control (tables 4,5). Protein concentration in the cytoplasm and nodal ganglion nuclei was definitely higher than the control, while at the same time, its level in superior cervical sympathetic ganglion neuron cytoplasm did not differ from that of the control.

Table 2

Volume of Bodies and Nuclei of the Nodal Ganglion Under Emotional Stress, mkm^3

	Number of Neurons	Number of Neuron Bodies	Number of Neuron Nuclei
Control	368	15,295±232	1486±34
Stress	319	12,938±270*	1316±34*

Table 3

Volume of Bodies and Neuron Nuclei of the Superior Cervical Ganglion Under Emotional Stress, mcm^3

	Mononuclear Cells		Dinuclear Cells			
	Number of Neurons	Volume of Neuron Bodies	Volume of Neurons	Number of Neurons	Volume of Neuron Bodies	Volume of Nuclei
Control	204	8840±221	960±35	130	10228±312	749±25
Stress	219	7530±208*	600±25	171	9664±280	561±28*

Table 4

Dry Weight of Cytoplasm and Neuron Nuclei of the Nodal Ganglion Under Emotional Stress, pg

	Number of Neurons	Neuron Cytoplasm	Neuron Nuclei
Control	368	2116±63	486±12
Stress	319	1955±63	443±13*

Table 5

Dry Weight of Cytoplasm and Neuron Nuclei of the Superior Cervical Ganglion Under Emotional Stress, pg

	Mononuclear Cells		Dinuclear Cells			
	Number of Neurons	Volume of Neuron Bodies	Volume of Neurons	Number of Neurons	Volume of Neuron Bodies	Volume of Nuclei
Control	204	1286±45	321±12	130	1337±61	250±9
Stress	219	1099±46	197±9	171	1325±58	181±28*

In other words, facts are presented which indicate that during emotional stress in rabbits predisposed to its development, changes in metabolic activity of both the sympathetic system and the vagus nerve structures occur. In the stellate ganglion and the sympathetic circuit (trunk), the change of metabolic activity takes place as a predominance of anabolic processes, indicated by the increased content of water-soluble protein, particularly important in the accomplishment of cellular functions, including providing for mediator synthesis. In the superior cervical ganglion, a reduction occurred of structural protein content in cytoplasm of mononuclear and dinuclear neuron nuclei, progressing against a background of maintaining watersoluble protein content at a normal level. The reduction noted in the nodal ganglion of watersoluble and structural proteins in nerve cell nuclei bears witness to the predominance there of catabolic processes (tables 6,7). This appears probable, inasmuch as the noted increases in protein content in the nuclei and cytoplasm of nodal ganglion neurons does not indicate a reduction in their rates of protein synthesis. The predominance of catabolic processes in the nodal ganglion may indicate a functional overstress of its structure.

Table 6

Protein Concentration in Cytoplasm and Nuclei of Nodal Ganglion Neurons Under Emotional Stress pg/mcm^3

	Number of Neurons	Cytoplasm	Nucleus
Control	368	0.152±0.003	0.319±0.003
Stress	319	0.170±0.004	0.341±0.006*

Table 7

Protein Concentration in Cytoplasm and Nuclei of Superior Cervical Ganglion Neurons Under Emotional Stress, pg/mcm^3

	Mononuclear Cells			Dinuclear Cells		
	Number of Neurons	Cytoplasm	Nucleus	Number of Neurons	Cytoplasm	Nucleus
Control	204	0.157±0.0033	0.325±0.004	130	0.146±0.004	0.331±0.004
Stress	219	0.160±0.004	0.330±0.004	171	0.153±0.005	0.321±0.006

Table 8

Correlation of LDG Isoenzyme Fraction Activity in Ganglia of the Autonomic Nervous System and Working Myocardium (4,6,7--number of animals)

LDG	Superior Cervical Ganglion		Nodal Ganglion		Stellate Ganglion		Sympathetic Circuit		Working Myocardium	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
	7	7	6	6	6	6	7	7	4	4
LDG_1	21.3	20.8	25.1	23.4	20.2	24.6	23.2	24.2	90.1	90.3
LDG_2	21.7	19.5	26.0	22.3	22.2	21.6	24.9	22.6	9.9	9.7
LDG_3	21.7	23.0	20.5	23.4	21.6	25.0	27.4	30.2		
LDG_4	21.8	23.0	18.7	21.4	23.5	18.5	18.2	18.2		
LDG_5	13.5	13.7	9.7	9.5	12.5	10.3	6.3	4.8		

It is possible that under conditions of stress, with the effect of the extraordinary stimulation, a rapid mobilization of the organism's reserves occurs, where energy in this instance is formed not only by the most effective means--oxidizing phosphorylation and glycolysis, but through a decomposition of proteins, possibly one of the reasons for the reduced protein content we observed. A reduced volume of nuclei and cytoplasm of nerve cells may be a consequence of reduced numbers of micromolecules.

It also occurred that there were no changes in the correlation of LDG isoenzymes in comparison to the control (table 8, figure 2) in homogenates of the superior cervical, stellate, nodal ganglia, and the sympathetic trunk of rabbits. The spectrum of LDG isoenzymes studied in the extramural ganglia of rabbits may be termed intermediate in type, which indicates that carbohydrate conversion in their nerve cells may take place as an anerobic glycolytic process as well as in aerobic reactions of the Krebs' cycle. Our observations might be compared with the data of M.A. Grebenkina (1952), who considered that the synthesis processes for acetylcholine in superior cervical ganglia neurons are dependent upon the oxidizing conversion of carbohydrates, whereas excitation of nerve cells in the transynaptic transmission of nerve impulses to a great degree is associated with glycolytic processes. An absence of shifts in the correlation of basic processes providing nerve cells of the ganglia with energy in rabbits under stress conditions does not preclude increased functional activity of structures of the autonomic nervous system, but bears witness to the great level of balance in their metabolism. It is known that only long-term (more than 8 hours) stimulation of the pre-ganglion fibers of the superior cervical ganglion in cats may result in a reduction of ATP content (Kalyunov, 1975). With short-term stimulation of the superior cervical ganglion, reduction of ATP content is achieved only after withdrawal from a perfused solution of glucose.

Table 9

Correlation of LDG Isoenzyme Fraction Activity in the Cardiac Conductive System (8,10--number of animals)

LDG	Control M±m	Stress M±m	t*
	8	10	
LDG_1	64.18±3.2	70.97±2.1	1.82
LDG_2	18.02±1.55	17.36±1.13	0.33
LDG_3	11.95±1.18	9.60±1.10	1.46
LDG_4	4.07±1.22	1.50±1.0	1.66
LDG_5	1.78±0.56	0.57±0.38	1.77

*t--Student's criterion of reliability

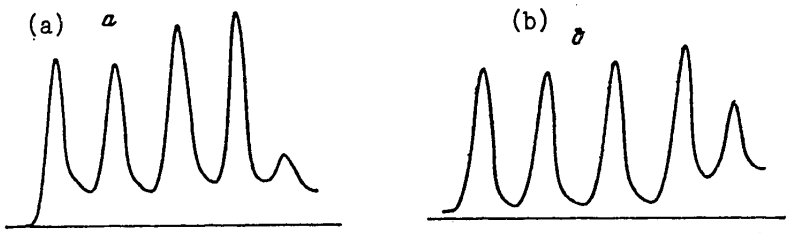

Figure 2. Spectrum of Lactate Dehydrogenase Isoenzymes of the Superior Cervical Ganglion

a--control, b--stress

In the study of rabbits' functioning myocardia, specifically those dying under stress conditions, changes could not be established in the correlation of myocardium LDG isoenzyme activities (table 8, figure 3). The spectrum of rabbit myocardium LDG isoenzymes indicates that in the cardiac muscle, the conversion of carbohydrates is accomplished by aerobic means. In those animals dying under stress conditions, there occurred a pronounced tendency toward changes in LDG isoenzyme correlations (table 9, figure 4). The correlation again occuring with isoenzyme fraction brought the LDG spectrum of the cardiac conductive system closer to the functioning myocardium LDG spectrum. An analysis of biochemical mechanisms underlying pernicious effects of emotional stress upon the heart, conducted in the laboratory of F.Z. Meyerson (1979), demonstrates the decisive role of glucocorticosteroids and catecholamines. Such a metabolic disorder of the myocardium is not related to reactions of the energy provision of the cardiac muscle, and ATP concentration in the myocardia of animals under stress does not change; the latter closely agrees with our observations on the maintenance of the normal LDG spectrum in the functioning myocardium of rabbits during emotional stress. A change of the LDG isoenzyme spectra of the conductive

system under stress indicates the close relationship of tissue metabolic processes and the functional state of the heart. We demonstrated that with the control animals, LDG isoenzyme spectrum of the conductive system is classified as cardiac, but relative to the spectrum of the functioning myocardium, includes more pronounced slow fraction (Figure 4). Stimulation of the negative emotionogenic zones of the hypothalamus resulted in changes in the conductive system's LDG spectrum: a growth was noted in the activity of the rapid "cardiac" isoenzymes. Thus, emotional stress is accompanied by changes in the course of energy processes in the tissues of the cardiac conductive system, and this must influence also its functional characteristics.

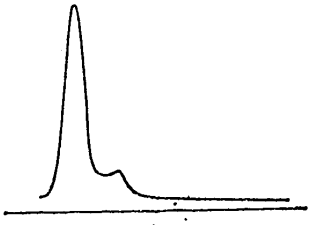

Figure 3. Myocardium Lactate Dehydrogenase Isoenzyme Spectrum of Control Animal

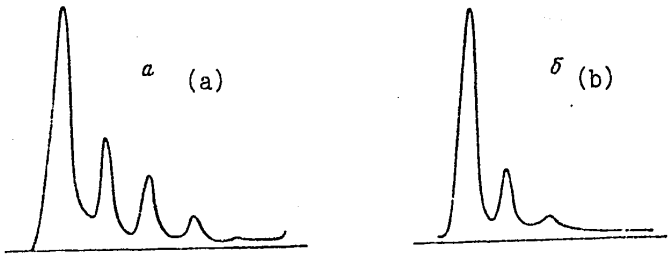

Figure 4. Cardiac Conductive System Lactate Dehydrogenase Isoenzyme Spectrum
a--control
b--stress

Data was obtained to the effect that not all individuals of the population of chinchilla variety rabbits develop identical cardiac-vascular disturbances with the stimulation of the negative emotionogenic zones of the hypothalamus. The nature of the changes in cardiac-vascular reactions permits the establishment of three groups of animals: stable, adaptive, and predisposed to the development of stress. This information is novel.

It was established that not all cellular formations of the peripheral autonomic nervous system are involved to the same degree in the stress process, with greatest changes detected in the neural structures of the nodal ganglion of the vagus nerve, where a disruption was established in the metabolism of proteins, that process being catabolic in nature, and conversely anabolic processes developed in neurons of the stellate ganglion and the sympathetic circuit, and the superior cervical sympathetic ganglion occupies an intermediate position. The observations cited indicate the preeminent role of the vagus nerve system in the development of acute experimental stress. The cardiac conductive system undergoes changes in the energy exchange; these shifts may be of considerable significance in the pathogenesis of emotional stresses of varying origin.

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ULTRASTRUCTURE OF CHICK EMBRYO SKELETAL MUSCLE TISSUE WITH MICROWAVE DAMAGE

Leningrad ARKHIV ANATOMII, GISTOLOGII I EMBRIOLOGII in Russian Vol 78, No 1, Jan 80 (manuscript received 16 May 79) pp 83-88

[Article by R. K. Danilov, Department of Histology (headed by Professor L. M. Kulagin), Kuybyshev Medical Institute imeni D. I. Ul'yanov]

[Text] Information regarding the effect of microwaves on the body of animals and man has been generalized in a number of summaries [Presman, A. S., 1965; Tolgskaya, M. S. and Gordon, Z. V., 1971; Minin, B. A., 1974]. However the structural-metabolic changes in the muscle tissues in microwave injury to the body have been covered extremely little in the literature, especially where modern research methods have been employed.

This work presents the results of a study on the effect of the energy of super-high frequency, of low-intensity electromagnetic field on the ultrastructural organization of the skeletal muscle tissue of developing chick embryos.

Material and Technique

In the first series of experiments, 16 chick embryos of the Legorn breed were irradiated on the seventh, eighth and ninth days of embryonal development. In the second series, 15 embryos were irradiated on the 12th, 13th and 14th days of development. Thirty unirradiated embryos of the same chick breed were the control. Chick eggs weighing 50±2.5 g were irradiated for 20 min with a superhigh frequency field with wavelength of 12.6 cm and power flux density (PFD) equal to 10 mW/cm^2. The microwave generator was a "Luch-58" unit. The PFD was controlled with the help of a "PO-1" instrument at a distance of 60 cm from the microwave emitter in the location plane of the study object.

The skeletal muscles (m. tibialis anterior) of the embryos were studied on the 12th, 16th and 19th days of incubation and on the 3rd day after hatching. Tissue fragments were fixed in a 2.5% solution of glutaric aldehyde for 2 h and additionally treated with a 1% solution of OsO_4 for 1 h. The fixatives were prepared in a 0.1 M phosphate buffer with sucrose (pH 7.4). The specimens were dehydrated in alcohol-acetone and poured into araldite. Sections were prepared on an LKB-III ultratome, contrast-stained with uranyl acetate and lead, and examined under a UEMV-100 K electron microscope. Specimens from three-nine animals for each period of study were used for stereometric analysis. Morphometry of the cellular organoids was done with a Weibel system [Weibel, E. R., et al., 1966; Kiseleva, Ye. V., et al., 1974]. The following were analyzed in the muscle elements: volumetric density of the mitochondria located on the

periphery of the myon ($V_{v\ mx\ п}$) and centrally ($V_{v\ mx\ ц}$), the volumetric density of the myofibrils ($V_{v\ mф}$), the volumetric density of the lipids ($V_{v\ лип}$), the surface area of the mitochondria ($S_{v\ mx\ п}$, $S_{v\ mx\ ц}$) and the numerical density of the mitochondria ($N_{v\ mx\ п}$, $N_{v\ mx\ ц}$).

Study Results

In the first group of irradiated embryos, on the 12th day of incubation, individual muscle fibers were found in the skeletal muscle tissue whose myofibrils occupied a greater area of the myon as compared to the control, and whose cross striation of the myofibrils was poorly pronounced. The mitochondria in these myons were smaller and the cristae were destroyed. On the whole, the structure of the muscle tissue did not differ significantly from the control.

In the skeletal muscle tissue of the 16-19-day old irradiated embryos, expansion of the perinuclear spaces in the myosatellites and the muscle fibers, and the presence of different-sized vacuoles in the sarcoplasm were observed. The nuclei of the muscle fibers contained large nucleoli and had numerous protrusions of the karyolemma. This indicates their hyperfunctioning. The sarcoplasm contained a large number of spiral polysomes and glycogen granules. The mitochondria were swollen. Some of them had destroyed cristae (fig 1,a). Disintegration of the myofibrils was defined in individual myons. The altered muscle fibers were adjoined by myosatellites. Their number was greater as compared to the control (see fig 1,b). When contracted muscles were fixed, the sarcolemma of the myons formed numerous protrusions.

In 3-day-old irradiated chicks, a number of muscle fibers contained a large number of fatty inclusions (see fig 1,c). The mitochondria formed accumulations in the center of the fiber. Myosatellites adjoined the altered myons. At the site of contact between the myosatellites and the muscle fiber, vesicle-formation and ruptures of the cytolemma were observed. This is associated with the merging of the myosatellite and the myon.

In the second group of embryos that were irradiated on the 12th-14th day of development, a set of ultrastructural changes in the muscle fibers was found that was similar to the first group. However, the processes of destruction were less pronounced. The compensatory-adaptive reactions of the muscle and other tissues in the muscle as an organ were traced more clearly.

Myosatellites with expanded perinuclear spaces (see fig 1,d) were defined in the muscles of the 16-19-day-old embryos of the second series of experiments. The peripheral mitochondria of the myons were swollen, with partially destroyed cristae. Individual myons had sections of ceraceous necrosis of the myofibrils. The skeletal muscles of the 3-day-old chicks had numerous polysomes in the peripheral part of the myons and large mitochondria with electron-dense matrix (fig 2,a). The cytoplasm of the capillary endotheliocytes contained a large quantity of pinocytic vesicles. This may indicate in favor of activation of transcapillary transfer of macromolecular compounds (see fig 2,b).

The data from stereometric study of skeletal muscles in the control and second group of embryos are given in the table. In the myons of the skeletal muscles of 16-day-old irradiated embryos, an increase was noted in the volumetric myofibril

density by 32%, and of the lipids 9-fold as compared to the control. The volumetric density of the mitochondria was diminished but not statistically reliably. The surface area of the peripheral mitochondria was diminished reliably. The numerical density of variously localized mitochondria was almost doubled as compared to the control.

Morphometric Indicators of Developing Skeletal Muscle Tissue of Control and Irradiated Chick Embryos, $\bar{x} \pm s_{\bar{x}}$

Morphometric indicators	Age of subject					
	Embryos of 16th day of development		Embryos of 19th day of development		3-day-old chicks	
	Control	Experiment	Control	Experiment	Control	Experiment
$V_{v\ m\phi}$ (%)	47±2.3	62±6 P<0.02	79±4	83±1,4	66±6	67±1.9
$V_{v\ mx.п}$ (%)	4.7±0.7	3.2±0.8	4±0.5	2.3±0.3 P<0.01	4.6±0.8	4±0.3
$V_{v\ mx.ц}$ (%)	3.6±0.6	2.7±1	5.3±0.1	4.8±0.5	4.5±0.8	3.7±0.3
$V_{v\ лип}$ (%)	0.02±0.005	0.18±0.01 P<0.001	0.35±0.01	0.7±0.1 P<0.01	0.1±0.01	3.4±0.4 P<0.001
$S_{v\ mx\ п}$ ($мм^{-1}$)	484±37	363±46 P<0.05	439±33	291±31 P<0.01	585±100	502±39
$S_{v\ mx\ ц}$ ($мм^{-1}$)	349±27	357±42	644±46	560±47	721±120	448±44 P<0.05
$N_{v\ mx\ п}$ ($мм^{-1}$)	215±26	405±61 P<0.01	245±20	180±24 P<0.05	271±46	256±28
$N_{v\ mx\ ц}$ ($мм^{-1}$)	295±57	615±83 P<0.01	381±34	384±36	393±62	259±24 P<0.05

Note. The P values are indicated for those results that differ from each other with 95% and greater probability.

By the 19th day of development, the muscle fibers of the irradiated and control embryos did not differ reliably in myofibril content. However, in the experiment, the volumetric density diminished reliably by 43%, the surface area by 34%, and the numerical density of the subsarcolemma mitochondria by 26% as compared to the control. The lipid content doubled in the myons of the irradiated embryos. The centrally located mitochondria changed quantitatively little.

In the 3-day-old experimental chicks, the volumetric lipid density tripled in the muscle fibers. The surface area diminished reliably by 38% and the numerical density by 34% of the centrally located mitochondria.

Discussion of the Findings

The conducted study shows that with irradiation by a SHF field of nonthermogenic intensity of chick embryos, the set of ultrastructural changes in the myons of the first group of embryos is more pronounced than in the second group. This may be associated with the developmental features of the skeletal muscle tissue. The seventh-ninth days of development of the chick embryos are characterized by a

transition from myoblastic to myosymplastic stage of myogenesis. In this period, the primitive nerve-muscle synapses appear for the first time [Atsumi, S., 1971; Kikuchi, T. and Ashmore, C. R., 1976]. The 12th-14th days of embryonal development are a period of intensive myofibril genesis occurring under nerve control. The greater sensitivity of the early stages of myogenesis (stage of myosymplasts and myotubes) to SHF-irradiation is apparently associated with reconstruction of the intracellular processes for synthesis of myofibrillar proteins and the lack of sufficient nerve-trophic effect. The more pronounced sensitivity of the myotubes to the effect of radiation as compared to the myoblasts and muscle fibers has been described in the works of A. A. Klishov (1971).

In addition to the processes of destruction, reactive-restorative processes are found in the skeletal muscle tissue which occur on the intracellular and cellular-tissue levels of organization. They are expressed as hyperplasia and hypertrophy of the organoids, activation of protein synthesis, and increase in the number of myosatellites. The myosatellites are probably less sensitive to the damaging effect of the SHF-field by the myogenic elements. When the myosatellites merge with the damaged muscle fiber, the nuclear apparatus of the myon is restored and the reparative processes are improved since the nuclear-cytoplasmic ratio increases.

In embryos of 16-day development the content of myofibrils increases in the myons. This reflects the intensification of protein synthesis in response to the effect of the SHF-field. This agress with the previously conducted study on protein biosynthesis in muscle tissue of irradiated chick embryos using labeled amino acids and cytospectrophotometry [Danilov, R. K. and Yegorova, L. I., 1977]. Intensification of protein synthesis in the myons is accompanied by a doubling in the numerical density of mitochondria. Increase in the number of mitochondria in the cells, according to the data of I. S. Nikol'skaya and L. I. Radzinskaya (1976), and N. D.Ozernyuk (1978) indicates intensification of the load and rise in oxygen consumption.

From the 16th to the 19th day of development, the rates of accumulation of contractile proteins in the muscles of the irradiated chick embryos is considerably lower than in the control. The content of myofibrillar proteins in the muscle tissue in the control and in the experiment are equalized. In 19-day-old experimental embryos, the subsarcolemma mitochondria suffer the most strongly in the myons. This is probably associated with the heterogeneity of differentiation of the central and peripheral mitochondria during the development of the muscle elements. According to the data of W. Müller (1976), the peripherally located mitochondria of the muscle fiber react differently to the load and are distinguished from the interfibrillar by a number of morphological and biochemical properties. The work of N. D. Ozernyuk (1978) has shown that the mitochondria of the peripheral zone of oocytes are more differentiated because of the intensive influx of oxygen from the oocyte surface. According to the studies of L. A. Kopteva et al. (1972), in the myocardium the variously localized mitochondria are distinguished by the rate of oxygen absorption, inclusion of labeled amino acids, and functional activity.

Consequently, reduction in the rate of protein accumulation, decrease in the quantitative indicators for the peripheral mitochondria reflect the depression of intracellular processes which is apparently a consequence of the insufficiently developed vascular system of the muscles and deteriorated supply of oxygen to the myons.

By the third day of development of the irradiated chicks, the morphometric parameters of the peripheral mitochondria approach the control values. The quantitative data of the myons of irradiated chick embryos diminish in the central mitochondria. This is associated either with the disrupted consumption or supply of interfibrillar mitochondria with oxygen, or change in the enzyme systems of the mitochondria. Deterioration in the intracellular bioenergetic processes results in fatty regeneration of part of the myons. The damaging effect of microwaves on oxygen consumption by the tissues is shown in the work of P. S. Neelakantaswamy (1978). Changes were not noted in the properties of the mitochondria themselves during irradiation. A special study of the respiratory control in isolated irradiated mitochondria with PFD of 10 mW/cm^2 did not reveal a disorder in oxidizing phosphorylation [Elder, J. A. and Ali, J. S., 1975].

Thus, the compensatory-adaptive response to SHF-irradiation includes reactions that occur on different structural levels of muscle organization: intracellular, cellular-tissue and organ. In this case, the interaction and interrelationship of cellular and cell-tissue processes are traced, as well as the vascular and nerve components of the muscle organ.

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EFFECT OF 50 Hz FREQUENCY ELECTROMAGNETIC FIELD ON CELL PASSAGE OF CERTAIN MITOTIC CYCLE PERIODS

Kiev TSITOLOGIYA I GENETIKA in Russian Vol 15, No 4, Jul-Aug 81
(manuscript received 11 Feb 80) pp 19-22

/Article by A. S. Panchuk, V. S. Kachura and V. D. Dyshlovoy, Kiev Medical Institute/

/Text/ In previous studies we substantiated the importance of the investigation of the biological effect of an industrial frequency electromagnetic field and reported data on the degenerative processes and changes in mitotic activity in cultures of fibroblast-like cells of human embryos subjected to the effect of this factor detected by us /1-4/. This work is a continuation of the study of the nature of changes in cell passages of mitotic cycle periods ensuing under various regimes of the effect of an industrial-frequency electromagnetic field with 50 kV/m voltage.

Material and methods. Initially trypsinized cultures of embryonal fibroblast-like human cells grown on cover glass by the standard method served as the object of investigation. Culture tubes were placed in an industrial-frequency electromagnetic field. The latter was created between the plates of a condenser placed in a thermostatically controlled chamber. H^3-thymidine of specific activity of 4.3 k/mmol in the dose of 1 μCi per ml of nutrient medium was introduced into the culture medium. On completion of incubation the preparations were fixed, covered with liquid emulsion of the P or M type, exposed at 4^0C, developed with an amidol developer and stained with Mayer's hematoxylin. The index of labeled nuclei was determined at the count of 1,000 cells and the number of granules of reduced silver, above 100 nuclei. The results obtained were subjected to statistical processing on the Mir-1 computer.

Results of investigations and their discussion. The index of labeled nuclei, intensity of DNA synthesis and length of S and G_2 periods were determined as the criteria of evaluation of the speed of cell passage of mitotic cycle periods. In all, six series of experiments on 42 cultures were set up. The time of onset of changes under the effect of an industrial-frequency electromagnetic field with 50 kV/m voltage was determined in the first series. For this purpose H^3-thymidine

was introduced into the culture medium, culture tubes were placed in the field and after 0.5, 3, 6, 12 and 24 hours the index of labeled nuclei was determined in the experiment and control. Investigations have shown (table 1) that 3 hours after the beginning of the effect there is a reduction in the speed of rise in the index of labeled nuclei. However, only at the end of the 24-hour period of effect the differences between the experiment and control reach a statistically significant level ($P<0.001$).

Table 1. Changes in the Index of Labeled Nuclei in Cultivated Fibroblast-Like Cells of Human Embryos Under the Effect of an Industrial-Frequency Electromagnetic Field With 50 kV/m Voltage

(1) Время воздействия ЭМП п/ч, ч	Индекс меченых ядер, проц. (2) (3) Контроль	(4) Опыт	n	t	P
0,5	13,3+1,3	13,3+0,9	10	0,26	>0,1
3,0	17,6+0,8	17,0+0,5	10	0,32	>0,1
6,0	20,7+0,4	20,3+0,9	10	0,51	>0,1
12,0	25,5+1,2	22,5+1,0	10	2,00	>0,05
24,0	36,0+0,7	31,6+0,1	10	7,70	<0,001

Key:
1. Time of effect of an industrial-frequency electromagnetic field, hours
2. Index of labeled nuclei, percent
3. Control
4. Experiment

Apparently, this points to the existence of a certain dependence between the manifestation of changes and the length of the field's effect.

The subsequent series of experiments were devoted to a verification of this assumption. The possibility of observing the reversibility of the ensuing changes and the speed of reducing reactions was also clarified.

Thirty minutes after a 5-hour period of stay of cultures in the field (the second series of experiments) the number of labeled nuclei in the experiment is bigger than in control (24.6 and 19.1% respectively, $P<0.001$). Subsequently the number of cells entering into the period of DNA synthesis increases at a lower speed in the experiment and after 20 hours the index of labeled nuclei does not differ in the experiment and control (table 2).

Thirty minutes after a 48-hour effect of an industrial-frequency electromagnetic field the index of labeled nuclei is 27.3% lower in the experiment than in control ($P<0.001$), that is, with an increase in the time of stay of cultures in the field the manifestation of changes rises. However, the ensuing changes are reversible and 3 hours after the cessation of irradiation the differences between the experiment and control become statistically insignificant ($P>0.05$) (table 3).

Table 2. Change in the Index of Labeled Nuclei in Cultivated Fibroblast-Like Cells of Human Embryos After a 5-Hour Effect of an Industrial-Frequency Electromagnetic Field With 50 kV/m Voltage

(1) Время воздействия ЭМП п/ч, ч	(2) Индекс меченых ядер, проц. (3) Контроль	(4) Опыт	n	t	P
0,5	18,1+1,4	24,9+1,3	11	3,1	<0,001
3,0	27,3+1,3	29,7+1,6	11	1,02	>0,05
8,0	35,1+2,5	36,5+2,1	10	0,01	>0,1
20,0	40,6+2,3	40,7+2,6	10	0,02	>0,1

Key:
1. Time of effect of an industrial frequency electromagnetic field, hours
2. Index of labeled nuclei, percent
3. Control
4. Experiment

Table 3. Changes in the Index of Labeled Nuclei in Cultivated Fibroblast-Like Cells of Human Embryos After a 48-Hour Effect of an Industrial-Frequency Electromagnetic Field With 50 kV/m Voltage

(1) Время выдерживания после прекращения воздействия ЭМП п/ч, ч	(2) Индекс меченых ядер, проц. (3) Контроль	(4) Опыт	n	t	P
0,5	7,1+0,4	5,1+0,9	10	6,1	<0,001
3,0	8,2+1,6	7,0+1,1	10	0,6	>0,1
6,0	10,7+1,2	9,6+0,9	10	0,8	>0,1
12,0	17,2+0,8	17,0+0,6	9	0,2	>0,1
24,0	27,0+1,3	27,3+1,6	9	0,1	>0,1

Key:
1. Time of observation after the cessation of the effect of an industrial-frequency electromagnetic field, hours
2. Index of labeled nuclei, percent
3. Control
4. Experiment

In the fourth series of experiments cultures were subjected to the field's effect for 7 days. Every 1 or 2 days in the experiment and control the index of labeled nuclei was determined after the field's effect during a 30-minute exposition with H^3-thymidine. Investigations have shown (table 4) that with an increase in the

time of stay of culture in the field the index of labeled nuclei remains reduced almost by the same magnitude during all the investigated periods. A direct relationship between the length of the field's effect and reduction in the number of cells entering into the phase of DNA synthesis has not been detected.

Table 4. Change in the Index of Labeled Nuclei in Cultivated Fibroblast-Like Cells of Human Embryos After Different Periods From the Beginning of the Effect of an Industrial-Frequency Electromagnetic Field With 50 kV/m Voltage

(1) Время воздействия ЭМП пром. ч	(2) Индекс меченых ядер, проц.		n	t	P
	Контроль (3)	Опыт (4)			
48	6,7+0,8	4,0+0,5	12	9,0	<0,001
96	4,8+0,4	3,3+0,8	12	5,3	<0,001
120	6,4+0,5	4,0+0,7	12	8,8	<0,001
144	6,8+0,7	5,0+0,6	12	6,1	<0,001
168	7,7+0,9	5,2+0,3	12	8,3	<0,001

Key:
1. Time of effect of an industrial-frequency electromagnetic field, hours
2. Index of labeled nuclei, percent
3. Control
4. Experiment

At the same time, as shown earlier [4], after a 96-hour effect of an industrial-frequency electromagnetic field the mitotic activity of cultures increases. Apparently, such an increase in mitotic activity in cultures during their long stay in the field is not due to the stimulation of cell passages of mitotic cycle phases, but is connected with the transition of part of the cells from the G_2-period to mitosis in connection with the removal of contact inhibition during the death of cells.

The slowing down of cell passage of mitotic cycle phases in response to the damaging effect of certain factors can be not only the consequence of a block of cell transition from one cycle phase to another (as, for example, under the effect of colchicine), but is also the result of prolongation of some phases as a result of the inhibition of metabolic processes in the cell. In order to detect the characteristics of the effect of an industrial-frequency electromagnetic field on the mitotic cycle of cultivated human cells, we determined the length of the S and G_2 periods, as well as the intensity of inclusion of H^3-thymidine in the nuclei of culture cells subjected to the field's effect. Since Quastler's equation is not applicable for systems in which the number of cells entering into a certain mitotic cycle period is not constant, the length of the S period was determined by a saturation curve analysis according to the following formula:

$$I_s = \frac{I_0 \cdot t}{I_t - I_0} \quad [5].$$

The length of the S period calculated in this way was 11.2 hours in control and 6.8 hours in the experiment.

It was not possible to determine the nature of changes in the length of the G_2 period under the effect of an industrial-frequency electromagnetic field owing to its great variability in individual cultures. Both in the experiment and in control labeled mitoses appeared after 5 to 24 hours.

The intensity of DNA synthesis was judged by the number of grains of reduced silver over cell nuclei in the experiment and control. Cultures subjected to the effect of an industrial-frequency electromagnetic field contain more nuclei with a greater number of grains as compared with control, which can point to an acceleration by the field of the speed of inclusion of H^3-thymidine during DNA synthesis.

Conclusions. Thus, under the effect of an industrial-frequency electromagnetic field cell passage of mitotic cycle periods is disturbed in cultures of fibroblast-like cells of human embryos. The disturbances are not connected with a block of the transition of one phase into another, but are due to the prolongation of individual periods of the cycle, in particular the S period, owing to the field's effect on metabolic processes in cells.

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INVESTIGATIONS OF FLUCTUATIONS IN OPERATOR SENSORY SENSITIVITY

Moscow BIOFIZIKA in Russian Vol 26, No 2, Mar-Apr 81
(manuscript received 8 Feb 80) pp 368-371

[Article by K. S. Burdin and A. D. Sizov, Biology Faculty, Moscow State University imeni M. V. Lomonosov]

[Text] Apparatuses in which reading is the result of visual adjustment of the brightness of adjacent visual fields are widespread in chemistry, biology and medicine. Reading is usually performed on such apparatuses in the following way: after the specimen under study is positioned, the brightness of the adjacent visual fields is adjusted visually, and the reading is taken from the dial of the apparatus. Then the photometric balance is disturbed, and the entire cycle is repeated. The number of readings in measuring a given specimen is determined by the desired random error of the result of measurement. With a little experience in operating the production nephelometers of the Zagorsk optical mechanics plant, 10-13 readings per minute are easily obtained using this procedure. When a graduated curve was plotted for nephelometric observations, we repeatedly noted that among the results of several dozen readings performed by the above described procedure, several mean arithmetic values were reliably distinguished from one another at different times of day for the same specimen with a known concentration of a weighed substance. In order to determine whether these changes are due to variation in the properties of the graduated solution, the latter was expressed from the apparatus, and a series of several dozen readings of the zero position of the apparatus was then performed. Next, in order to simplify interpretation of the results, experiments were performed using the photometric head of the nephelometer alone without the nephelometric fitting. Two beams of light from a mat screen illuminated by an incandescent lamp connected with a stabilized power source fell on the photometric head. Adjustment of the light beams in an NFM [expansion unknown] nephelometer is conducted by measuring the brightness of one of the beams using a diaphragm with a variable area, a depiction of which is projected into the pupil of the observer's eye. Before measurement, the drum of the right diaphragm of the nephelometer was set at 90 percent with respect to the transmission dial and left in this position for the entire duration of the experiments. The left diaphragm was diverted from this value in the direction of the lowest values of the transmission factor, in order for disturbances in photometric balance to be easily detected visually. The brightness of the field of rotation of the left drum was then adjusted in the direction of the high values on the transmission scale, and readings were taken and recorded. Next, the

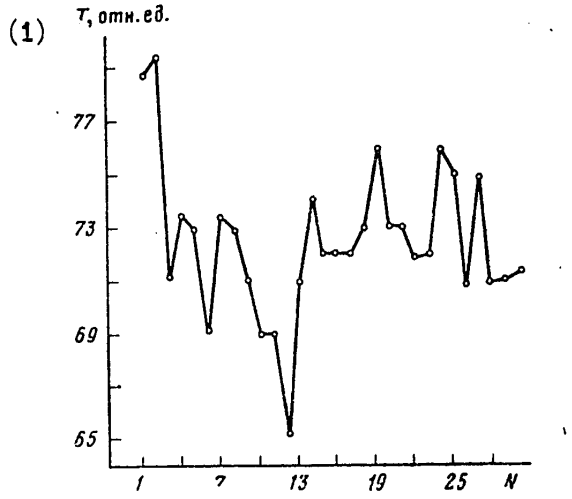

Figure 1. Dispersion of Readings of the Position of Photometric Balance at Reading Rate of 10-13 Readings Per Minute.

Key:
1. T, relative units

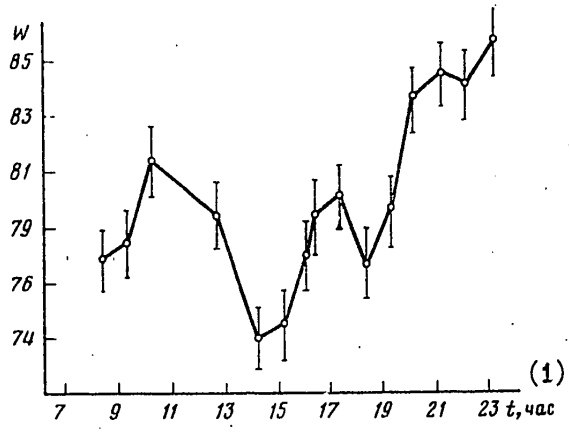

Table 2. Arithmetical Means From 30 Readings of the Position of Photometric Balance As a Function of Time of Day (Moscow Time). Confidence Intervals Indicated For Level of Significance of 5 Percent. Experiments Conducted 1 April 1979.

Key:
1. Hour

balance of the brightness of the visual fields was disturbed and readjusted, and readings were taken and recorded, etc. The results were plotted on a graph (fig 1) where the number of measurements is plotted along the abscissa and readings from the left drum of the nephelometer are plotted along the ordinate. We computed the mean arithmetic values of zero point of the apparatus (i.e., the position of photometric balance) at a given moment from the results of several dozen such readings. It turned out that the difference between the mean values of the zero position of the nephelometer without the specimen under investigation sometimes was statistically significant both at different times of the same day and at the same times of day but on different days. Figures 2 and 3 present some results of the experiments. The time interval between dates of measurements corresponding to figs 2 and 3 is 5 days. Altogether 117 separate experiments were conducted by the same operator over the course of 6 consecutive days.

The results obtained indicate that the causes of the observed fluctuation in the zero position of the nephelometer is due not to the apparatus but to the operator. These fluctuations have an effect on the position of the graduated curve relative to the ordinate axis and on the accuracy of determinations of the concentration of weighed substances performed using a nephelometer. The least dispersion in determining the position of the photometric balance is due to a time interval from 23:50 to 1:00 (later nocturnal hours were not investigated). A possible cause of the fluctuations of the zero position is the biological rhythms of man.

It should be noted that the principal underlying the operation of the reading apparatus of the nephelometer--visual adjustment of the brightness of adjacent visual fields--is related to the methods of determining the differential visual threshold (1). The data we obtained suggests that fluctuations in the sensitivity of the human operator's visual analyzer influence the results of measurements even in work with production models of apparatuses. This influence introduces additional uncontrolled error into the results of determinations. Consequently, when the data of nephelometric determinations are compared, the results of analyses conducted by an operator performing measurements in order to plot a graduated curve are more comparable. More than 40 years ago in a series of investigations on adaptation by the eye in peripheral vision, P. P. Lazarev described fluctuations in sensitivity as a function of the time of day, season and location on the retina (2). He also touched upon the question of the influence of geophysical factors on adaptation (2) and developed the thermal theory of geographic influences on the cerebrum (2). Now, concerning this question, it is impossible not to take into consideration the vast factual material on the influence of penetrating geophysical factors on the atmosphere and biosphere of Earth which was amassed during the past decades (3, 4). A logical development of our experiments is study of the correlation in simultaneous determinations of the status of the photometric balance by several independent operators in connection with changes in geophysical factors in addition to those which P. P. Lazarev investigated in his time. In this connection, V. Ye. Zhvirblis's recent work (5) on investigation of fluctuations observed during visual adjustment of the polarimeter to maximum transmission must be remembered. It seems to us that these results belong to the class of phenomena (at least in the part of the experiments where visual recording is used) caused by fluctuations in human sensory sensitivity, but the methodical bases of the experiments were described by Fekhner, P. P. Lazarev and others in their time. Among the recent publications,

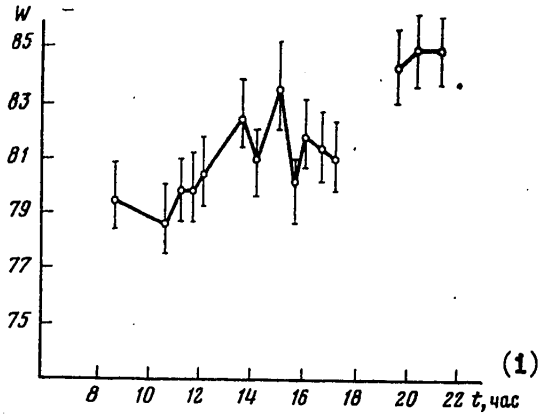

Figure 3. Same, But Date of Experiment Is 6 April 1979

Key:
1. Hour

monograph (1) discusses this subject in detail. Consequently the original interpretation V. Ye. Zhvirblis proposed for the phenomena he observed seems to us to be indisputable. The principal difference between our positions is that according to V. Ye. Zhvirblis' interpretation, an external factor influences the symmetry of the polarimeter, i.e., the apparatus, disturbing it. Here, the role of man is reduced to merely ascertaining this fact. Our data on the nephelometer without the use of polarized light indicates, rather, that the receiver of exogenous disturbances is, in fact, man and not the apparatus. The fact that the effects described by V. Ye. Zhvirblis are easily demonstrated on simple polarimeters only when operated at maximum transmission, but that precision polarimeters (with reading accuracy of up to +0.0001 degree) are required in order to observe them at minimum transmission, has a simple explanation if we assume that the culprit for the described effects is not the apparatus but the human operator. For this, it is natural to assume in working with the polarimeter that when the Nicol prisms are parallel, the accuracy of reading the angle of rotation of the apparatus dial proves to be higher than the threshold of sensitivity for the human visual analyzer. Accordingly when the dial is rotated within a certain range close to the maximum transmission of the polarimeter the difference in the brightness of adjacent visual fields is not visually perceptible. In essence, the method used by V. Ye. Zhvirblis is extremely close to the one we used in this study and to the method of determining human sensory sensitivity. This method, which is well known in psychology, was developed more than 100 years ago by Fekhner and is known as the "standard deviation method," as one of three classical psychophysical methods. It may be hoped that experiments investigating correlations between fluctuations of sensory sensitivity and variation in the intensity of penetrating geophysical factors will give further grounds for forming opinions on the validity of correction of established concepts. The results of the experiments of this sort conducted will be published.

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PROBLEMS OF SPACE BIOLOGY, VOL 40: BIOLOGICAL EFFECTS OF ELECTROMAGNETIC RADIATION IN MICROWAVE RANGE

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[Annotation, introduction and table of contents from book "Problems of Space Biology. Vol 40: Biological Effects of Electromagnetic Radiation in the Microwave Range", by Vsevolod Vasil'yevich Antipov, Boris Il'ich Davydov and Viktor Semenovich Tikhonchuk, Department of Physiology, USSR Academy of Sciences, Izdatel'stvo "Nauka", 1150 copies, 221 pages

[Text] This monograph sums up and analyzes extensive material from the literature, as well as the authors' experimental data on the biological and biophysical effects of electromagnetic radiation in the microwave range. The authors studied the quantitative correlations between biological effects and radiation density, time of exposure and type of biological object on different levels of organization--cellular, systemic, organismic--in different species of animals--mice, rats and dogs.

This book is intended for specialists concerned with radiobiology, aviation and space medicine.

There are 6 tables, 69 illustrations; bibliography supplied on 29 pages.

Introduction

The intensive development of science and technology in the last 5-10 years has resulted in a significant increase in number of various instruments and equipment, which are the source of nonionizing electromagnetic radiation (EMR). In this regard, the radiation burden on man increased and continues to grow. This applies in particular to such fields as aviation and cosmonautics, where even now the density of radiation output [power?] is sometimes many times higher than the existing standards. The prospect of development, in particular, of radar technology, will be related to further increase in the radiation burden.

Nonionizing electromagnetic radiation has become one of the significant environmental factors. For this reason, it is logical for medical men, biologists and engineering physicists to be very interested in studying the biological effects of EMR, mechanisms of action, determination of permissible levels of exposure, questions of dosimetry, etc.

The increased interest in this problem is indicated, in particular, by extensive discussion thereof at the 4th International Congress on Radiation Protection in France (Paris, April 1977), international symposiums on the effects of electromagnetic waves in the United States (Arly, October 1977) and Finland (Helsinki, August 1978), and others.

In the last 10 years, many comprehensive surveys have been published (Petrov, Subbota, 1966; Guy, Lehmann, 1974; Michaelson, 1975), as well as monographs (Gordon, 1966; Presman, 1968; Petrov, 1970; Baranski, Czerski, 1976, and others), which deal with analysis of experimental and clinical data on mechanisms of biological effects of EMR, questions of setting standards, ways and means of protection against radiation, etc., attention being focused chiefly on EMR in the radiofrequency range.

Our objective here was to summarize and submit to critical analysis the existing data in the literature of an experimental and clinical nature, concerning the biological effects of EMR, as well as to discuss our own findings on the biological effects of microwave EMR. We tried to find approaches and criteria that would enable us to assess quantitatively and prognostically the main patterns of damage to the organism.

In view of the fact that exposure to EMR during space flights is associated with the set of various flight and space factors, in particular ionizing radiation, we deemed it purposeful to discuss some theoretical aspects of the problem of the combined effect of flight factors and specific experimental data concerning interaction between EMR and gamma rays.

Critical analysis of literature sources and our own research was made from the standpoint of quantitative radiobiology, on the conception of "energy interaction" between EMR and biological objects. We tried to examine the quantitative functions between biological effects and density of radiation power, time of exposure and type of biological object on different levels of organization--cellular, organic, systemic and organismic--in experiments on three species of animals (mice, rats and dogs). We believe that this approach enabled us to make a comparative analysis of the biological effects of nonionizing and ionizing EMR.

While we adhere to the conception of "energy interaction" between EMR and biological objects, we do not reject the possible "information interaction," formed in the course of evolution. As we know, such interactions are characterized by transformation of information, transmission, coding and storage thereof. The biological effects due to these interactions may not depend on the energy added to a given system, but nature of information.

The first chapter of this book offers a brief description of the biophysical bases of interaction between EMR and matter and, mainly, biological systems. The second describes comparative analysis of biological effects of EMR in critical systems of an organism--nervous, reproductive, visual and hemopoietic. The third chapter describes and analyzes data on the lethal effects of EMR. Some patterns of effects of EMR on the integral organism of mammals are discussed in the fourth chapter. The fifth chapter describes some reactions of the neuroendocrine system, mainly the adrenals. Finally, the last chapter of the monograph discusses the biological effects of combined flight factors, including the results of studies of biological interaction between microwave EMR and ionizing radiation. The book ends with a conclusion and bibliography, which lists all works that the authors could find on the subject through 1979.

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